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14. ABSTRACT

(Taken From Initiating PI Report)
Prostate cancers that recur after so-called androgen ablation therapy ('CR-CaP') are typically more aggressive, more likely to spread to local lymph nodes and bones, and less likely to respond to second-tier treatments, and therefore, contribute to significantly decreased patient survival. We posit that enzymes called Src-family kinases (SFK) are required for the progression to CR-CaP, and thus, targeting these enzymes should prevent CR-CaP formation to suppress their growth. We will use animal models of human and mouse CR-CaP in conjunction with genetic and biochemical experiments to show that SFK are critical to the formation of CR-CaP, and thus, are therapeutically targetable using SFK-specific drugs. Our important pre-clinical studies on the critical role played by SFK in CR-CaP disease will serve as the foundation to establish immediate clinical trials in which CaP patients are treated with drugs such as KX2-391 at the commencement of androgen-deprivation therapy.

15. SUBJECT TERMS

Src, androgen receptor, castration recurrent, CWR22, Lyn-shRNA, TRAMP

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Introduction

From Initiating PI:

We are studying the role of Src-family kinases (SFK) in promoting castration-recurrent prostate cancer (CR-CaP) using genetic and pharmacological approaches along with several animal models of CR-CaP. Our synergistic collaboration is based the expertise of the initiating PI (Gelman) in the molecular signaling of SFK in cancer progression, combined with the expertise of the partnering PIs in the CWR22 and TRAMP CR-CaP mouse models (Mohler and Smith, respectively), and in the role of neuroendocrine cells (NE) in the progression of CR-CaP (Smith).

Body

My role has been to assist Dr. Gelman who had very little knowledge of the CWR22 model with the experiments described under task 1, task 2, and task 5 specifically as well as the general conduct, data analysis, and reporting of the experiments described in all 9 tasks.

***Task 1*. Produce CWR22 cells with tetracycline-regulated Src or Lyn-shRNA expression**

I provided methodologies that were used by Dr. Gelman via the RPCI Mouse Tumor Modeling Resource (MTMR) to grow androgen-stimulated CWR22 tumor xenografts in male nude mice implanted with sustained release testosterone pellets. When these tumors were 250 mm^2, they were removed and converted into single cell suspensions using methodology we developed. The single cell suspensions were infected with a titer of control or Src-shRNA lentiviruses containing surrogate GFP marker and the cells maintained their expression of GFP for 3 passages *in vitro*.

***Task 2.* Inject SCID mice with CWR22 Src- or Lyn-shRNA (or vector) cells**

Dr. Gelman's research has been facilitated by the evolution of his knowledge regarding the androgen-dependent CWR22 human prostate cancer xenograft model. Last year, CWR22 primary xenografts were ready to receive the Src- and Lyn-shRNA lentiviruses by infecting the cells *in vitro* and then reinjecting them subcutaneously reinjecting 10^6 cells subcutaneously with Matrigel into new testosterone-pelleted castrated male nude mice. Infected cells and controls have been placed into groups of 20 mice each and the mice were castrated (testosterone pellet removed) when the primary tumor reached 250 mm^3. There was a small (but statistically significant) decrease in the rate of primary tumor regression in the group receiving the Src minus vs. the control shRNA. The mice were monitored for tumor recurrence for 8 months and 6 of 12 control shRNA tumors recurred after 148 ± 18 days whereas only 3 of 12 Src-shRNA tumors recurred after 160 ± 14 days. Recurrent tumors retained their strong lentivirus encoded GFP expression but Src knockdown was lost in the Src-shRNA recurrent tumors.

***Task 3.* Test whether KX2-391 (vs. vehicle or Dasatinib) can prevent recurrent AI-CaP or NE malignancy**

The methodologies developed under task 1 and 2 were applied in task 3 where mice were treated with KX01, Dasatinib or vehicle. T-pelleted, castrated male nude mice were injected with CWR22, their pellets removed and 28-day treatments of KXO1, Dasatinib or vehicle administered. These mice were monitored for tumor recurrence for 6 mo. KXO1 or Dasatinib decreased to 4 of 20 (20%) or 5 of 20 (25%) animals compared to vehicle where 10 of 20 (50%) of animals suffered recurrence. Time to recurrence was also delayed. Toxicity was minimal.

***Task 5.* Demonstrate increased NE proliferation in recurrent AI-CaP (CWR22) or NE (TRAMP) lesions**

The CWR22 recurrences from the control of KXO1 or Dasatinib-treated groups exhibited similar frequency of neuroendocrine cells upon immunostaining. However, the possibility that neuroendocrine cells provide the nidus for recurrence of prostate cancer in the androgen- dependent CWR22 human prostate cancer xenograft model requires tissue from the androgen-stimulated original tumor, the regressing tumor, the recurring tumor, and the castration-recurrent tumor that kills the mouse. The CWR22 tissue microarray originally constructed at the University of North Carolina more than 10 years ago has been exhausted. In order to perform the studies proposed in Task 5, tissue was procured from laboratory mice baring the androgen-dependent CWR22 xenograft at all steps of regression, progression, and gross recurrence. A new experimental tissue microarray has been

constructed where each tumor represented in the tissue microarray (see table) has stored corresponding frozen tumor, frozen benign prostate, and frozen serum. This biorepository of tissue will facilitate the conduct of the experiments proposed in Task 5. Immunostaining of the tissue microarrays constructed will be performed.

CWR22 Tissue Microarray Project

	N	tumor tissue	mouse prostate	serum
Human benign prostate	1	n		
Human CaP	1	y		
Human colon cancer	1	y		
Intact mouse ventral prostate	1	n		
Intact CWR22	8	y	y	y
1 day CX	2	y	y	y
2 day CX	2	y	y	y
4 day CX	2	y	y	y
6 day CX	4	y	y	y
6 day CX + TP 48 h	4	y	y	y
12 day CX	4	y	y	y
12 day CX + TP 48 h	4	y	y	y
12 day CX	2	y	y	y
30 day CX	2	y	y	y
60 day CX	2	y	y	y
90 day CX	2	y	y	y
120 day CX	4	y	y	y
Recurrent	8	y	y	y
Recurrent + TP 48 h	4	y	y	y
Recurred from CWR22	6	y	y	y

Key Research Accomplishments

- successful efficient transduction of primary androgen-dependent CWR22 tumor cells with shRNA-encoding lentiviruses
- re-injection of testosterone pelleted, castrated nude mice with transduced primary CWR22 cells
- Proof of principle that SFC knockdown using lentiviral delivered shRNA decreases the frequency of, and time to, recurrence after ADT transduced primary CRW22 cells to determine the time course of response
- establishment of a biorepository of CWR22 specimens that spans the continuum from androgen-stimulated tumor to castration-recurrent tumor
- demonstration of a decrease in the frequency of recurrence of CWR22 after castration by treatment with KXO1 or Dasitinib.

Reportable Outcomes

A manuscript, “Src controls castration-recurrence of CWRR22 prostate cancer xenografts” has been prepared for submission for publication.

Conclusion

The project is progressing at pace with no major obstacles. The synergistic component of the award has been critical to our success thus far and to our projected ability to proceed with the outstanding tasks. This project could not have been accomplished by each of the individual labs. Specifically, this project is progressing strictly because of the combining of the various expertise, such as the active use of the CWR22 and TRAMP models, the isolation and identification of NE cells, and the development and use of the shRNA-encoding lentiviruses. The PIs have also had scheduled meetings to strategize and to review data.

References

None

Appendices
None